Ultraviolet inactivation and excision-repair in bacillus subtilus

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SUMMARY

This thesis consists of a collection of 5 papers dealing with ultraviolet inactivation and excision-repair in the transformable bacterium **Bacillus subtilis**, specially with respect to transforming DNA. One of the main objectives was to study UV inactivation of a number of transforming markers. To that purpose, a multiple auxotrophic mutant, strain 8G-5, was constructed, which served as the recipient strain. The procedure of isolation and the characterization of strain 8G-5 are described in paper I. This strain transforms quite satisfactory; single marker transformation frequencies of a few percent are usually obtained. Although the various markers are slightly different with respect to efficiency of transformation, they probably belong to only one class, all being of the high-efficiency type. The markers were located relative to each other by means of marker frequency analysis. Of the 8 markers studied, $\mathbf{trp}_2$ and $\mathbf{tyr}_1$ are closely linked in transformation. The **rib** marker was shown to be weakly linked to the $\mathbf{trp}_2$-$\mathbf{tyr}_1$ pair of markers. No further linkages exist between the markers in strain 8G-5.

UV inactivation of the 8 markers is described in papers II and III. DNA isolated from prototrophic cells was irradiated with various doses of UV and subsequently used to transform 8G-5 recipient cells. The results demonstrate that the various markers are inactivated at different rates. Our working hypothesis was that differential marker inactivation is due to differences in the frequency of potential photochemical lesions in the marked segments of DNA. However, interpretation of the results of our marker inactivation experiments according to this hypothesis was complicated for at least two reasons. Firstly, the results show that marker sensitivities to UV depend on the DNA concentration during transformation. At saturating amounts of DNA, the markers are apparently more sensitive to UV than at non-saturating amounts. This is probably due to the fact that at saturating amounts of DNA, in addition to the molecules carrying selected markers, more non-selected damaged molecules are integrated into the recipient genome than at non-saturating amounts. Therefore, more potential transformants are killed under the former conditions, which results in overestimation of marker sensitivities. This complication was avoided by determining the marker sensitivities to UV at DNA concentrations far below saturation. Secondly, complications will also arise, if after uptake by recipient cells, the inactivated markers are repaired to different degrees. To avoid this complication, an excision-repair-deficient derivative was isolated from strain 8G-5. The properties of this strain are also described in paper I. This strain, 8G-11, is unable to excise pyrimidine dimers, to reassume DNA synthesis after UV irradiation, and to reactivate UV-inactivated bacteriophage. As compared to strain 8G-5, it transforms normally with unirradiated DNA, but poorly with UV-irradiated DNA. Because of these properties strain 8G-11 can be

classified as being deficient in excision-repair (**uvr**$^-$). When **in vivo** photoreactivation of UV-irradiated donor DNA is prevented by working under dim light, and excision-repair by using strain 8G-11 as recipient, differential marker inactivation by UV of 254 nm still exists (paper II), which supports the hypothesis that the frequency of potential photochemical lesions determines the sensitivity to UV of the marked segments of DNA.

Comparison of the results on marker inactivation obtained with strain 8G-5 and strain 8G-11 allowed us to compute the fraction of inactivating lesions which is not repaired by the 8G-5 (**uvr**$^+$) recipient. The size of this fraction is marker-specific and varies from 13% to 26%, indicating that excision-repair is not equally efficient for different markers in irradiated DNA. On the average about 80% of the induced lesions are succesfully repaired.

The involvement of $\hat{T}\hat{T}$ dimers in both differential marker inactivation and differential marker repair was studied in the following way. Transforming DNA was irradiated with 313 nm light in the presence of acetophenone as sensitizer. Under these conditions exclusively $\hat{T}\hat{T}$ dimers should be induced[42,43]. However, our results (presented in paper III) demonstrate that, in addition to $\hat{T}\hat{T}$ dimers, non repairable single-stranded DNA breaks are also produced. **In vitro** photoreactivation of the $\hat{T}\hat{T}$ dimers in the irradiated DNA allowed us to compute the relative contributions of the dimers and the single-stranded DNA breaks to total marker sensitivities. Although only one break occurs per approximately 90 $\hat{T}\hat{T}$ dimers, it appears that the breaks account for about 20% of the observed marker sensitivities.

Differential marker inactivation and differential marker repair were also observed in the acetophenone system of irradiation. The results demonstrate that, irrespective of the **uvr** genotype of the recipient, the single-stranded DNA breaks inactivate the various markers to the same extent. From this observation it can be concluded that in the acetophenone system differential marker inactivation is exclusively due to differences in the frequency of $\hat{T}\hat{T}$ dimers in the marked segments of DNA. In addition, differential marker repair is exclusively due to differences in the efficiency of repair of $\hat{T}\hat{T}$ dimers. Support for the conclusion that differences in the frequency of $\hat{T}\hat{T}$ dimers in the marked segments of DNA cause differential marker inactivation is that photoreactivation experiments indicate that excellent correlation exists between the frequency of $\hat{T}\hat{T}$ dimers in the marked segments of DNA and the sensitivity of the markers to irradiation in the acetophenone system. Further support for this conclusion is provided by the observation that, as judged from heat denaturation studies, markers relatively rich in thymine usually are relatively sensitive to irradiation and, conversaly, that markers relatively poor in thymine usually are relatively resistant to irradiation.

Comparison of the results obtained in the acetophenone system with those obtained in the 254 nm system of irradiation, shows that both the relative orders of marker sensitivities and of marker repairabilities are closely similar in the two systems of irradiation. We consider this as evidence that also in the

254 nm system of irradiation $\hat{TT}$ dimers are the main cause for differential marker inactivation and that differences in the efficiency of repair of $\hat{TT}$ dimers are the main cause for differential marker repair.

The fate of UV lesions in transforming DNA was studied more detailed in the experiments described in paper IV. The principal aims were to investigate: firstly, genetic integration of UV-irradiated transforming DNA, and secondly, the kinetics of **in vivo** repair of UV-inactivated transforming DNA. The effect of UV lesions in transforming DNA on genetic integration was studied in the following way. UV-irradiated DNA was used to transform a repairless **uvr**$^-$ recipient. After allowing time for integration to occur, DNA was reextracted and subsequently assayed on **uvr**$^+$ and **uvr**$^-$ second recipients for the presence of dark-repairable damage in recombinant type molecules. The presence or absence of dark-repairable damage in the recombinant type molecules was judged by the number of recombinant type transformants obtained on the **uvr**$^+$ relative to the number on the **uvr**$^-$ second recipient. The results show that transforming DNA irradiated with moderate doses of UV (2,000 erg/mm^2) is integrated almost equally efficiently as non-irradiated DNA, and that integrated UV lesions account almost exclusively for the inactivation. However, irradiation of the DNA with 20,000 erg/mm^2 of UV results in a significant exclusion of damaged DNA from the process of integration. Under these conditions, the contribution of the exclusion to total marker inactivation amounts to approximately 10% in **uvr**$^+$ and to less than 1% in **uvr**$^-$ recipients.

The kinetics of **in vivo** excision-repair of UV-irradiated transforming DNA were studied by a closely similar method. In this case, a repair-proficient **uvr**$^+$ strain served as the first recipient in transformation with UV-irradiated DNA. The DNA was reextracted at various intervals after mixing cells and donor DNA, and the residual amount of dark-repairable damage in recombinant type molecules in the reextracted DNA was determined as a function of time of reisolation. The results indicate that repair only starts after the donor DNA is integrated into the recipient genome. Completion of repair requires from 20-45 min for DNA irradiated with 2,000 erg/mm^2, and approximately 2 h for DNA irradiated with 15,000 erg/mm^2. The results also indicate that UV-damaged eclipse phase DNA (DNA that is taken up but not integrated) reextracted from recipient cells, is not subject to excision-repair in **uvr**$^+$ second recipient strains.

The possibility of **in vitro** excision-repair of UV-inactivated transforming DNA was studied in the experiments described in the last paper of this thesis. The hypothesis put forward by KELLY **et al.**[40] that DNA polymerase performs both the excision and the replication step in excision-repair, prompted us to investigate whether UV-irradiated transforming DNA can be repaired **in vitro** by the combined action of UV-specific endonuclease, DNA polymerase and DNA ligase. The results show that the biological activity of inactivated DNA increases significantly following incubation with the three enzymes. The restored activity can not be further increased by photoreactivation **in vitro**, indicating that

photorepairable dimers are no longer present. It could be demonstrated that dimers are specifically excised upon incubation of the irradiated DNA with the three enzymes, and that under our conditions approximately 50% of the induced lesions are succesfully repaired. These results show that, at least **in vitro**, KELLY's[40] model applies to repair of UV lesions in DNA. Whether this model also applies to repair **in vivo**, remains to be established. It may also be envisaged that alternative pathways of excision-repair exist.